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Identification of Hazardous Organic Chemicals in Fish from the Ashtabula River, Ohio, and Wabash River, Indiana

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16. ABSTRACT

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Identification of Hazardous Organic Chemicals in Fish from the Ashtabula River, Ohio, and Wabash River, Indiana

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Composite fish samples from the Wabash River, IN, and the Ashtabula River, OH, were analyzed by conventional pesticide procedures for PCBs, DDTs, chlordane components, and hexachlorobenzene. Additional aliquots of each sample were processed by gel permeation chromatography and were analyzed with gas chromatography–mass spectrometry by using both electron impact and negative chemical ionization modes. These analyses resulted in the identification of a series of chlorinated alkanes, chlorinated alkenes, chlorinated alkylamines containing 2–5 carbons, and polychlorinated styrenes in the Ashtabula River sample, and a series of chlorinated norbornenes, pentachlorobenzyl alcohol, and pentachlorophenol in the Wabash River sample.

Biomonitoring is an essential part of hazard assessment for the regulation of industrial activity to ensure environmental protection. Basic research aimed at the identification and quantitation of trace levels of hazardous chemicals provides an accurate description of actual chemical residues existing in our environment. This type of information can then be used for establishing priorities in regulatory and health-related research activities. In addition, biomonitoring provides validation of predictions made from laboratory experiments. For instance, we have shown that the bioaccumulation potential for organic chemicals is readily estimated from structure (1), and that only highly lipophilic chemicals are likely to pose residue problems in fish when discharged into natural waters (2). Because fish tissue provides both a concentrated and integrated sample of lipophilic chemicals, multiclass residue analyses of fish from major watersheds offer a valuable means of detecting contamination by potentially hazardous chemicals. We describe here the application and results of recently developed analytical methods on fish samples from the Ashtabula River in Ohio and the Wabash River in Indiana.

Mass spectral analysis has the potential for rapid identification of chemical residues at the parts per billion level in tissue. The limiting factor is often the cleanup procedure used to isolate residues from natural lipids. Conventional cleanup procedures sufficient for analysis by electron capture gas chromatography (EC/GC) are often not sufficient for mass spectrometry. The high degree of selectivity and sensitivity of EC/GC for toxic chemicals, especially polychlorinated chemicals, tolerates natural lipids that interfere with the less selective electron impact mass spectrometry (EI/MS). We have taken 2 approaches to solving this problem. First, we have used gel permeation chromatography (GPC) to isolate toxic chemicals from fatty samples, yielding residues that are sufficiently free of interfering lipids for MS analysis (3). Second, we have used negative chemical ionization mass spectrometry (NCI/MS) (4) in addition to EI/MS for compound identification. The advantages of NCI over EI are increased sensitivity and selectivity for electronegative chemicals (5). In addition, we have used the selected ion summation (SIS) technique (6), an ion current profile of the summation of the intensities of selected ions that is generated if and only if all selected ions are observed above a set threshold, to rapidly screen full mass range scans of EI/MS and/or NCI/MS data prior to complete spectral data interpretations, library searches, and GC relative retention time calculations.

Experimental

Preparation of Samples

Fish samples were collected by State and Federal field staff and shipped frozen to the Environmental Research Laboratory, Duluth, MN. Fish samples were homogenized to provide a composite sample of whole fish. Subsamples of 20 and 100 g tissue were blended with anhydrous Na_2SO_4 to dry the tissue and were extracted ex-

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haustively in a Soxhlet extractor with hexane–methylene chloride (1+1).

The 20 g sample extract was concentrated and placed on a 20 g Florisil column. The column was eluted with 250 mL hexane and the eluate was concentrated to 100 mL for EC/GC quantitation of major contaminants such as PCBs.

The 100 g sample was concentrated and diluted to a lipid concentration of 100 mg/mL methylene chloride for cleanup by GPC (3). The extract was cleaned up by sequential elution of 5 mL aliquots on a 60 cm × 2 cm bed of SX-2 Bio-Beads (Bio-Rad Laboratories) with methylene chloride as the solvent. The combined 160–225 mL fraction containing the chemicals of interest from each injection was concentrated to 5 mL, injected on an identical GPC column, and eluted with methylene chloride–cyclohexane (1+1) to further isolate the residues from the lipids. Again the 160–225 mL fraction was collected and concentrated for GC/MS analysis.

Electron Capture Analysis

Quantitative analysis of 20 g sample extracts for PCBs, DDTs, and hexachlorobenzene was performed on a Hewlett-Packard Model 5700A gas chromatograph equipped with a Model 3352B laboratory data system and a linear argon–methane detector. Instrument parameters and operating conditions are as follows: glass column, 2 m × 2 mm id packed with 80–100 mesh Gas-Chrom Q coated with a mixture of 4% SE-30 and 6% OV-210; column temperature programmed from 160 to 200°C at 2°/min followed by 8 min hold at 220°C; carrier gas, mixture of 95% argon and 5% methane flowing at 30 mL/min. The chromatograms for PCB analysis were interpreted by a computer program previously described (4).

In addition to packed column EC/GC, all samples were screened on a Hewlett-Packard 5700A glass capillary column EC/GC system using splitless injection. Instrumental parameters are as follows: glass capillary column, 30 m × 0.25 mm coated with SE-30; column temperature programmed from 100 to 225°C at 4°/min followed by 30 min hold at 225°C; helium carrier gas flowing at 30 cm/s.

GC/MS Analysis

The 100 g samples were screened by a flame ionization detector (FID) and then subjected to mass spectral analysis on either Varian MAT CH-5 or Finnigan 4000 interfaced to INCOS data system.

Instrumental parameters for CH-5 system are as follows: glass column, 2 m × 2 mm packed with 80–100 mesh Gas-Chrom Q coated with 3% OV-101; column temperature programmed from 100 to 225°C at 4°/min followed by 30 min hold at 225°C; helium carrier gas flowing at 25 mL/min; electron energy 70 eV; source temperature 300°C; helium separator, dual stage fritted-glass effusion.

Instrumental parameters for the Finnigan 4000 are as follows: glass capillary column, 30 m × 0.25 mm coated with SE-30; column temperature programmed from 100 to 225°C at 4°/min followed by 30 min hold at 225°C; helium carrier gas flowing at 30 cm/sec; electron energy 70 eV; source temperature: EI 300°C, NCI 100°C; source pressure for NCI: 0.5 torr of methane.

Multiple ion detection (MID) was used on the Finnigan 4000 in the electron impact mode for quantitation of components of technical chlordane in the 20 g samples. The following ions were monitored: m/z 373, chlordane; m/z 409, nonachlor; m/z 272, heptachlor; m/z 387, oxychlordane; m/z 355, heptachlor epoxide; and m/z 442, decafluorotriphenylphosphine, used as the internal standard. These ions are the most intense ions above m/z 250 in the electron impact mass spectrum of these compounds.

The Finnigan 4000 was equipped with a Grob injector set for a 30 s delay during injection. The source and quadrupole rods were tuned to obtain a spectrum of the reference compound decafluorotriphenylphosphine (Ultra Mark 443) that matched published spectra (7).

Reagents and Standards

Florisil (Fisher Scientific) was washed in a Soxhlet extractor (hexane–acetone (1+1)) for 24 h, dried in a vacuum oven for 24 h, and activated at 600°C for 12 h.

Sodium sulfate (Fisher Scientific) was treated in the same manner as Florisil.

Hexane, acetone, methylene chloride, and cyclohexane (Burdick & Jackson, pesticide grade) were redistilled.

Analytical standards were obtained from Health Effects Research Laboratory, Environmental Toxicology Division, Environmental Protection Agency, Research Triangle Park, NC 27711; Chem. Services, Inc., West Chester PA; and PolyScience Corp., Evanston, IL. Octachlorostyrene was obtained from Dow Chemical Co., Walnut Creek, CA.

Results and Discussion

Residue analysis of a wide variety of chemicals from a complex substrate such as fish tissue requires highly efficient cleanup techniques that do not discriminate between classes of chemicals. A GPC system of Bio-Rad SX-2 beads eluted with a mixture of methylene chloride–cyclohexane efficiently separates both polar and nonpolar chemicals from interfering

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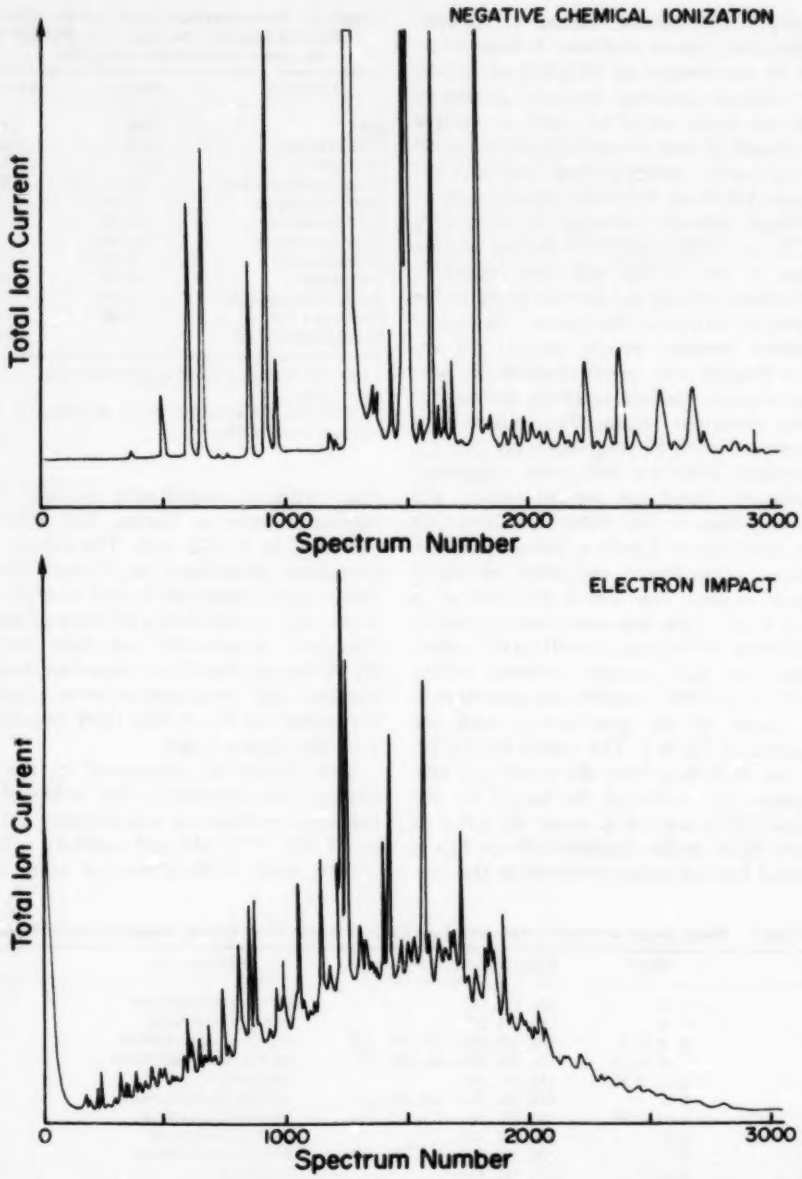

Figure 1. Total ion current profiles for negative chemical ionization data (upper) and electron impact data (lower) obtained on a Finnigan 4000 glass capillary GC/MS system for Ashtabula River fish sample.

lipids. This system yields nonpolar chemicals such as hexachlorobenzene (HCB) and *p,p'*-DDE as well as 3,4-dichloroaniline; recoveries are generally greater than 90% (3).

Negative chemical ionization was used to complement EI GC/MS data. This technique has the advantages of high sensitivities for chlorinated chemicals and low sensitivities for

interfering hydrocarbons, similar to conventional electron capture analyses. A direct comparison of sensitivities of NCI/MS to EI/MS is very difficult however, because sensitivity depends on many variables, such as sample matrix, length of time of operation in a specific ionization mode, source tuning, and NCI reagent gas. We have generally experienced an approximate 100-fold increase in sensitivity for HCB, *p,p'*-DDE, and OCS during routine operation in first EI/MS and then NCI/MS, using the instrumental parameters listed in the Experimental section of this paper. The use of high purity methane as the reagent gas resulted in electron attachment as the major ion-forming process, yielding molecule anions and therefore molecular weight data. These data were matched with EI fragmentation data by GC retention time for unknown compound identifications. Total ion current profiles for EI and NCI data on the Ashtabula River fish sample, presented in Figure 1, demonstrate the differences in analytical capability of the 2 ionization modes. The actual presence of a chemical in a sample was confirmed by one or both of these techniques, as well as GC retention time and GPC elution volumes, before either GC or GC/MS quantitation was started.

The results of the quantitation work are summarized in Table 1. The values for DDTs, PCBs, and HCB have been discussed in a previous paper (2). Although the values for the PCBs and DDTs are not unusual, the value of 3140 ppb HCB in the Ashtabula River fish is the second highest value observed in the previous work, exceeded only by fish from the Altamaha River at Darien, GA, which were measured at 11 600 ppb. The values for the chlordane components in Wabash River fish reflect trends generally found in major watersheds, that is, low levels of most of the major chlordane components and their metabolites (8). Although chlordane, nonachlor, heptachlor epoxide, and oxychlordane were identified in the Ashtabula River fish, their concentrations were less than 0.5 ppb.

The chlorinated compound of the second highest concentration in the Ashtabula River fish was identified as octachlorostyrene (OCS) by EI/MS, NCI/MS and capillary column retention time. Polychlorinated styrenes have

Table 1. Concentrations (ppb) of polychlorinated chemicals found in the fish of the Wabash River, IN, and the Ashtabula River, OH

Chemical	Wabash	Ashtabula
DDT	310[a]	130[a]
PCB 1016/1242	1010[a]	4460[a]
PCB 1254	2200[a]	2740[a]
Hexachlorobenzene	30[a]	3140[a]
trans-Chlordane	8.54[b]	<0.50[b]
cis-Chlordane	12.98[b]	<0.50[b]
trans-Nonachlor	20.04[b]	<0.50[b]
cis-Nonachlor	4.89[b]	<0.50[b]
Heptachlor	<0.50[b]	<0.50[b]
Heptachlor epoxide	<0.50[b]	<0.50[b]
Oxychlordane	<0.50[b]	<0.50[b]
Octachlorostyrene	<0.50	500[a]

[a] EC/GC analysis, limit of detection 0.5 ppb, analysis error ±2%.
[b] MID GC/MS analysis, limit of detection 0.5 ppb, analysis error ±8%.

Table 2. Major polychlorinated chemicals found in Ashtabula River fish by electron impact GC/MS

RRT[a]	Ions used for SIS searches	Compound
0.12	106, 108, 110	dichloropropadiene
0.13	131, 133, 135	tetrachloroethane
0.39, 0.44	190, 192, 194 – 155, 157, 159	tetrachlorobutadiene
0.46, 0.51	224, 226, 228 – 189, 191, 193	pentachlorobutadiene
0.53, 0.70	143, 145, 147	unknown
0.57	258, 260, 262 – 223, 225, 227	hexachlorobutadiene
0.58, 0.65	260, 262, 264 – 177, 179, 181	hexachlorobutene
0.67	214, 216, 218	trichlorobenzene
0.70	226, 228, 230 – 191, 193, 195	pentachlorobutene
0.70	179, 181, 183	unknown
0.73	276, 278, 280	hexachloropentane
0.76, 0.82	243, 245, 247 – 131, 133, 135	pentachlorobutylamine
0.82, 0.74	277, 279, 281 – 165, 167, 169	hexachlorobutylamine
0.85	248, 250, 252	pentachlorobenzene
1.00	282, 284, 286 – 247, 249, 251	hexachlorobenzene
1.03, 1.06	308, 310, 312 – 273, 275, 277	hexachlorostyrene
1.13	342, 344, 346 – 307, 309, 311	heptachlorostyrene
1.19	287, 289, 291	unknown
1.28	376, 378, 380 – 341, 343, 345	octachlorostyrene

[a] Relative retention time determined on CH-5 GC/MS system; hexachlorobenzene = 1.00.

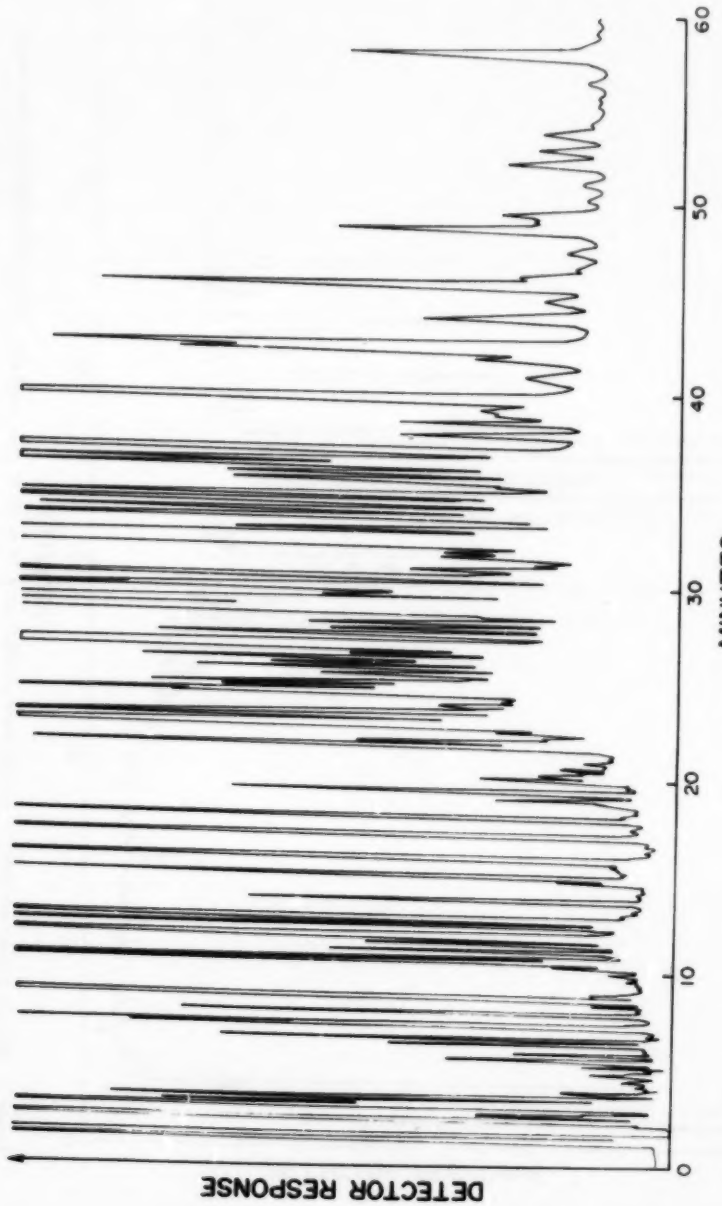

Figure 2. Electron capture gas chromatographic trace obtained on a 30 m SE-30 glass capillary column for Ashtabula River fish extract.

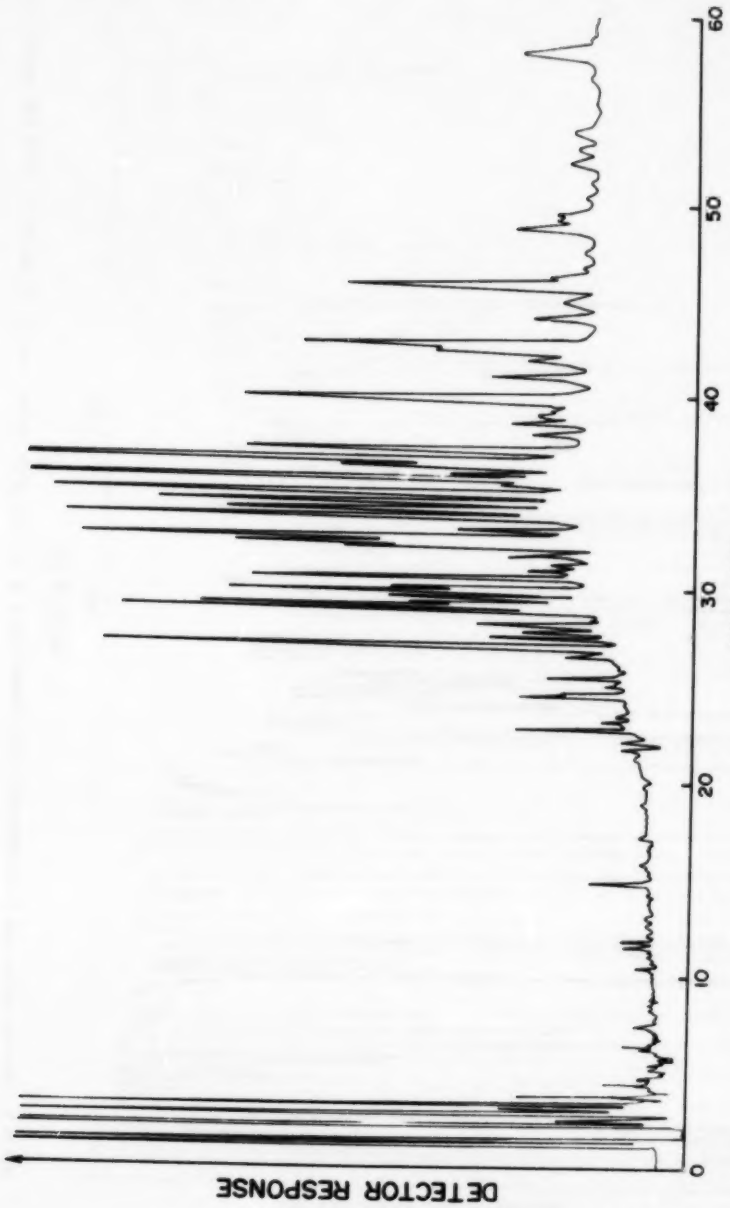

Figure 3. Electron capture gas chromatographic trace obtained on a 30 m SE-30 glass capillary column for Wabash River fish extract.

Table 3. Polychlorinated chemicals found in Wabash River fish by electron impact GC/MS

RRT[a]	Ions used for SIS searches	Compound
0.83	261, 263, 265	hexachloronorbornadiene
0.85	248, 250, 252	pentachlorobenzene
0.93	235, 270, 297, 332	heptachloronorbornene
0.96	278, 280, 282, 243, 245, 247	pentachlorobenzyl alcohol
0.99	235, 270, 297	unknown
1.00	282, 284, 286	hexachlorobenzene
1.03	259, 261, 263, 214, 216, 218	hexachloroepoxynorbornene
1.08	301, 303, 305, 336, 338, 340	chlordene
1.12	264, 266, 268	pentachlorophenol
1.12	370, 335, 270, 235	heptachlor
1.40	261, 263, 265	endrin

[a] Relative retention time determined on CH-5 GC/MS system; hexachlorobenzene = 1.00.

been found previously in Great Lakes fish by Kuehl et al. (9). In Figure 1, the NCI total ion current profile of the Ashtabula River fish, the peak at spectrum 1795 is OCS. A concentration estimate based on ion abundances observed in the NCI data is 1.0 ± 0.5 ppm, while EC/GC analysis was 500 ppb. In addition to OCS, 2 isomers of hexachlorostyrene and 2 isomers of heptachlorostyrene were also observed. The ions used for SIS searches of GC/MS data, and GC relative retention times of the chlorostyrenes are given in Table 2.

In addition to the chlorostyrenes, the Ashtabula River fish contained a series of polychlorinated hydrocarbons of 2–6 carbons listed in Table 2. Figure 2, an EC/GC trace of this sample, shows the complexity and relative intensities of these compounds. Another class of compounds identified was polychlorinated alkylamines, specifically pentachloropropylamine, pentachlorobutylamine, and hexachlorobutylamine. The butylamines were identified by EI GC/MS and confirmed by NCI GC/MS, and the propylamines (2 isomers) were detected by NCI GC/MS. The additional compounds observed at m/z 143, Cl_3; m/z 179 Cl_4; and m/z 287, Cl_5 were not identified.

The EC/GC chromatogram of the Wabash River fish is shown in Figure 3. Although the lack of volatile chlorinated compounds may appear to make this sample less interesting than the Ashtabula River sample, GC/MS data have identified a number of unusual chemicals (Table 3). Among these are hexachloronorbornadiene, heptachloronorbornene, and hexachloroepoxynorbornene. These chemicals have previously been identified in fish from the Mississippi River as intermediates and metabolites of intermediates used in the production of endrin(10). Endrin has also been identified in the Wabash samples. In addition, a compound showing a heptachloronorbornene-type of fragmentation pattern was observed at relative retention time 0.99 (HCB = 1.00); however, this compound could not be positively identified.

Two additional compounds identified were pentachlorophenol, a chemical widely used particularly as a wood preservative, and pentachlorobenzyl alcohol, the origin of which is not known at this time (2).

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